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Intestinal ciliate composition found in the feces of the Cypriot wild donkey, *Equus asinus* Linnaeus, 1758

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Abstract

Species composition and distribution of large intestinal ciliates was investigated in the feces from 13 Cypriot wild donkeys, free-living in the Karpaz national park, Northern Cyprus. We identified 16 ciliate genera and 22 species. This is the first report on intestinal ciliates in Cypriot wild donkeys, and no endemic species were observed. The genus *Cycloposthium* occurred in all animals. The mean number of ciliates was $3.0 \pm 2.5 \times 10^4$ cells ml^{-1} of feces and the mean number of ciliate species per host was 6.5 ± 4.8. Characteristics of the wild donkey ciliates was almost identical to those reported in other equids from various regions around the world. We thus conclude that there is no pronounced geographic variation in the intestinal ciliate fauna of equids.

Keywords: Intestinal ciliates; Cyprus; Wild donkey; *Equus asinus*; Feces

Introduction

Since the intestinal ciliates were first reported by Gruby and Delafond (1843) from the large intestine of the domestic horse, the intestinal ciliate fauna of equids has been surveyed intensively (Gassovsky 1919; Hsiung 1930; Ozeki et al. 1973; Strelkow 1929; Strelkow 1939). It is now well known that many ciliate species are excreted alive in the feces of equids (Ike et al. 1981, 1983a, b; Imai et al. 1999; Ito et al. 1996; Tung 1992). The ciliates invade the host by oral ingestion (coprophogy) and then settle in its large intestine (Ike et al. 1985).

Although the composition of the intestinal ciliate community of various equids is known in general, no investigations have been conducted on the ciliate fauna in the Cypriot wild donkey, *Equus asinus* Linnaeus 1758. Cypriot wild donkeys are free-living in Karpaz National Park, which is located on an isolated island in the Mediterranean Sea off the northern coast of Cyprus. The aim of this study was to identify and quantify the fecal ciliate fauna from those animals on the island and compare the data with previous studies on equids from various other locations.

Materials and methods

Fecal samples were collected from 13 individuals of the Cypriot wild donkey (*Equus asinus* Linnaeus, 1758) located in the Karpaz national park in Northern Cyprus. The samples were collected from July 2007 up to April 2008. Karpaz national park covers an area of 11.000 hectares. Its vegetation consists of *Pancreatium*

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maritimum, *Cakile maritima*, *Limonium albidum* and *Pistecia lentiscus* (Göçmen et al. 2008). The fecal samples were collected immediately after defecation and fixed and stained in about 2 times as much methyl-green formalin saline solution (MFS) as their original volume (Ito et al. 1996; Ogimoto and Imai 1981). This procedure was used to preserve the integrity of the cell and its internal structures. The MFS served as a nuclear stain and Lugol's iodine was used to stain skeletal plates. Fecal samples were sieved through 562.5-μm mesh gauze and kept in the dark until examination.

Total cell counts were made at 400× magnification with a Neubauer hemocytometer counting chamber. Differential counts of species were estimated from smear slides with a total of 50 to 60 cells identified for each species (Göçmen and Gürelli 2009). Details of the ciliate morphology were investigated at 1000x magnification using an oil immersion objective microscope.

Ciliate genera and species were identified and classified based mainly on the descriptions of Hsiung (1930), Kornilova (2003, 2004), Lynn (2008), Ozeki (1977), Strelkow (1939).

Results

Frequency of appearance (i.e. the number of hosts in which the species was detected/number of hosts examined) and the relative composition of genera and species are shown in Table 1. We identified 22 species belonging to 16 genera. The ciliate fauna consisted of 7 genera and 8 species of Buetschliidae, 3 genera and 6 species of Blepharocorythidae, 1 genus 1 species of Cycloposthiidae, 4 genera, 6 species of Spirodiniidae, and 1 genus and 1 species of Allantosomatidae.

For individual wild donkeys, the total number of species per animal ranged from 2 to 15, with an average of 6.5 ± 4.8 (SD).

The genus *Cycloposthium* was found in each animal, followed by *Blepharocorys valvata* and *Allantosoma intestinalis* (>60% frequency). The relative contribution to the total ciliate composition was >10% in *Cycloposthium* sp., *Allantosoma intestinalis*, *C. edentatum*, *B. valvata*, while it was low (<10%) in the other genera and species.

The average abundance of ciliates in the intestinal contents from the 13 Cypriot wild donkeys was $(3.0 \pm 2.5) \times 10^4$ cells ml^{-1}. Values ranged from 0.5×10^4 to 8.5×10^4 cells ml^{-1} (Table 2).

All cells of *Tetratoxum excavatum* Hsiung, 1930 observed in our study had smooth surfaces without longitudinal cuticular ridges in the side near the macronucleus and the opposite side of the body.

Large cells (237.6 ± 34.7 μm, n = 25) of *Cycloposthium edentatum* Strelkow, 1939 were found in 3 donkeys (23.1%), contributing 2.2 ± 5.1% to total ciliate numbers.

Discussion

In the present study, 22 ciliate species representing 16 genera were identified, but no novel or endemic species was detected. The number of identified taxa was very low in comparison to previous reports from other equids (Table 3). The only exception was the low taxon concentrations reported for the Japanese native tokara pony (Ito et al. 1996). However, no report is available on the ciliate density in the intestines of other donkeys and mules. Our results are in accordance with Strelkow's (1939) assumption that the majority of the intestinal ciliates of equids has a worldwide distribution, although the number of species was relatively low in the Cypriot wild donkeys. Strelkow (1939) classified *T. excavatum* into two morphotypes. One morphotype was characterized by longitudinal cuticular ridges on both the dorsal and ventral surfaces of the body (*T. excavatum f. excavatum*) and the other one by cuticular ridges on almost the whole body surface (*T. excavatum f. sulcatum*). We found only the former morphotype in our study, and only in one of the 13 Cypriot wild donkeys examined.

Hsiung (1930) reported a body length of *Cycloposthium edentatum* ranging from 146-230 μm, whereas Strelkow (1939) divided *C. edentatum* into four morphotypes based on its body surface and its body size. This author described large specimen of *C. edentatum* as *C. edentatum f. gigas* with body lengths of 200-290 μm. We observed two morphotypes of *C. edentatum* in the wild donkeys. The average body length of the smaller morphotype (*C. edentatum f. edentatum*) was 159.4 ± 17.2 μm (125.0-185.0 μm, n = 25). Mean body length of the larger morphotype (*C. edentatum f. gigas*) was 237.6 ± 34.7 μm (200.0-312.5 μm, n = 25), i.e. similar to Strelkow's study.

The lower ciliate abundance and diversity in the feces of the Cypriot wild donkeys could be caused by the reduced water content of their feces. When the feces are firm and dry, most protozoa become partially desiccated and destroyed (Fantham 1921). The varying consistency of the feces may be a result of geographical location, isolation from other equids, feeding habitat and food type differences. The length of the host's intestine may also play a role, because donkeys have shorter and thicker intestine than horses.

Ike et al. (1985) investigated the establishment of the ciliate fauna in foals, especially the mode of transmission of ciliates from one host to another. Their results showed that coprophagy from their mother's feces was the primary vector and that the newly established ciliate

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Table 1. Frequency of appearance* and percentage composition of intestinal ciliate genus and species in the feces of 13 Cypriot wild donkeys.

Genus/Subgenus/Species	Frequency of appearence (%)	Percentage composition (%)	
		Mean ± SD	Range
Bundleia	46.2	9.3 ± 12.7	0-37.1
postciliata (Bundle, 1895)	53.8	7.9 ± 11.5	0-38.7
Chlamydobundleia			
triangularis Strelkow, 1939	23.1	2.0 ± 3.9	0-10.8
Polymorphella	30.8	1.7 ± 3.6	0-12.5
ampulla (Dogiel, 1929)	30.8	1.7 ± 3.6	0-12.5
Ampullacula	15.4	0.4 ± 1.0	0-3.5
ampulla (Fiorentini, 1890)	15.4	0.4 ± 1.0	0-3.5
Prorodonopsis	7.7	0.3 ± 1.2	0-4.4
coli Gassovsky, 1919	7.7	0.3 ± 1.2	0-4.4
Blepharosphaera	7.7	0.1 ± 0.4	0-1.6
intestinalis Bundle, 1895	7.7	0.1 ± 0.4	0-1.6
Blepharoconus	15.4	1.1 ± 2.8	0-8.1
benbrooki Hsiung, 1930	15.4	1.1 ± 2.8	0-8.1
Holophryoides	7.7	0.2 ± 0.6	0-2.2
ovalis (Fiorentini, 1890)	7.7	0.2 ± 0.6	0-2.2
Blepharocorys	84.6	23.0 ± 22.5	0-80.0
valvata (Fiorentini, 1890)	61.5	11.2 ± 21.5	0-80.0
angusta Gassovsky, 1919	53.8	6.7 ± 11.8	0-42.9
curvigula Gassovsky, 1919	30.8	3.3 ± 5.7	0-16.7
microcorys Gassovsky, 1919	30.8	1.8 ± 4.0	0-13.5
Charonnautes	7.7	0.4 ± 1.5	0-5.3
equi (Hsiung, 1930)	7.7	0.4 ± 1.5	0-5.3
Circodinium	7.7	0.3 ± 1.2	0-4.4
minimum (Gassovsky, 1919)	7.7	0.3 ± 1.2	0-4.4
Spirodinium	23.1	0.9 ± 1.9	0-5.4
confusum Hsiung, 1935	7.7	0.1 ± 0.4	0-1.6
uncinucleatum Hsiung, 1935	15.4	0.8 ± 1.9	0-5.4
Triadinium	23.1	0.6 ± 1.3	0-4.4
caudatum Fiorentini, 1890	23.1	0.6 ± 1.3	0-4.4
Ditoxum	15.4	0.5 ± 1.3	0-4.4
brevinucleatum Strelkow, 1931	15.4	0.5 ± 1.3	0-4.4
Tetratoxum	15.4	0.30 ± 0.8	0-2.2
unifasciculatum (Fiorentini, 1890)	7.7	0.2 ± 0.6	0-2.2
excavatum Hsiung, 1930	7.7	0.1 ± 0.6	0-2.2
Allantosoma	69.2	15.2 ± 17.2	0-52.6
intestinalis Gassovsky, 1919	69.2	15.2 ± 17.2	0-52.6
Cycloposthium	100	45.1 ± 28.6	12.9-100.0
sp.[a]	92.3	29.0 ± 19.2	0-66.7
edentatum, Strelkow, 1939	46.2	16.1 ± 25.3	0-76.9
Total	16 genera 22 species		

*The ratio of the number of hosts in which a species appeared divided by the total number of animals surveyed.
[a]Not identified for species due to degeneration.

fauna in foals is therefore strongly affected by their mother's ciliate fauna. Accordingly, the ciliate fauna would be passed down largely unchanged from parents to their young. Transmission of ciliates between hosts other than parent and offspring would appear difficult because in adult horses, ciliates are not likely to pass easily through the host's stomach (Imai et al. 1999). Possibly, digestive enzymes in the gastrointestinal tract of foals are less harmful for ciliates than in adult equids. If we assume that the ciliate fauna of a host has been established by inheritance from its ancestor, the similarity of the ciliate composition among the various

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Table 2. Abundance of intestinal ciliates in the feces of 13 Cypriot wild donkeys.

	Donkey no.												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Total ciliates ($\times 10^4$ cells ml^{-1}) Mean $\pm$ SD = (3.0 $\pm$ 2.5) $\times$ 10^4 cells ml^{-1}	2.5	2	0.5	8.5	4.5	0.5	1.5	2	1.5	5.5	1.5	2.25	6.8

Table 3. Total ciliate abundance and distribution of the total number of genera and species of the ciliates in the intestine contents of equids from various locations around the world.

Locality[a]	Total ciliates ($\times 10^4$ cells ml^{-1})	Range ($\times 10^4$ cells ml^{-1})	Total no. of genera	Total no. of species	References[b]
China	[d]	[d]	19	30	1
Japan	3.4[d]	[d]	19	40	2
Japan	9.0[d]	0.4-113.0	22	49	3
Japan	140.0[d]	[d]	23	50	4
Japan	1.4[d]	[d]	11	18	5
Middle Asia	[d]	[d]	25	57	6
Taiwan	38.1 $\pm$ 35.9[c]	0.3-127.0	19	38	7
Cyprus	3.0 $\pm$ 2.5[c]	0.5- 8.5	16	22	Present study

[a]Number of animals and breed: China (20 horse, donkey and mule); Japan (17 light horse); Japan (60 race horse); Japan (18 kiso horse); Japan (20 tokara pony); Middle Asia (184 kulan); Taiwan (40 riding horse); Cyprus (13 wild donkey).
[b](1) Hsiung (1935a, b, 1936); (2) Ike et al. 1981; (3) Ike et al. 1983a; (4) Imai et al. 1999; (5) Ito et al. 1996; (6) Kornilova 2003; (7) Tung 1992.
[c]Mean $\pm$ SD.
[d]Data not reported.

equids may indicate that the ciliate fauna has been stabilized in their hosts' intestines before the differentiation of the races of equids (Imai et al. 1999).

The origin of the Cypriot donkey is not known and it has been assumed that they were derived from the African wild ass (Hadjisterkotis et al. 2000). No fossils belonging to the pre-settlement area of Cyprus have been found so far, which supports the assumption that donkeys were brought to Cyprus from abroad as domestic animals.

In conclusion, the 22 species of intestinal ciliates recorded from the Cypriot wild donkey are also known from other equids in different locations. The ciliates detected in our study do not appear to have undergone a pronounced specific differentiation since they originally reached the island.

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